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RESEARCH ARTICLE

Hormesis-like growth and photosynthetic physiology of marine diatom *Phaeodactylum tricornutum* Bohlin exposed to polystyrene microplastics

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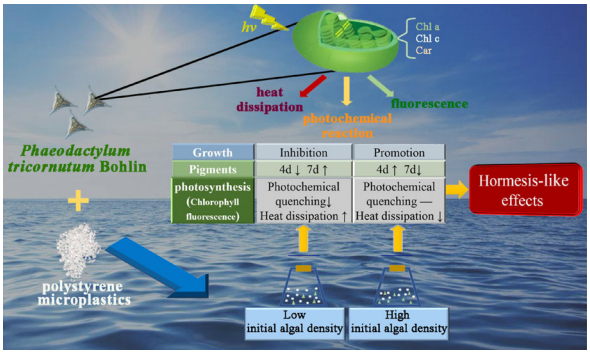
HIGHLIGHTS

- Polystyrene microplastic caused hormesis-like effects in *Phaeodactylum tricornutum*.
- Low concentration of microplastic promoted growth, otherwise the opposite was true.
- The change trends of pigment contents were opposite at two initial algae densities.
- The chlorophyll fluorescence parameters were more sensitive at low algae density.

GRAPHIC ABSTRACT



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ABSTRACT

The effects of pristine polystyrene microplastics (pMPs) without any pretreatment at different concentrations (0, 10, 20, 50, and 100 mg/L) on *Phaeodactylum tricornutum* Bohlin at two initial algae densities (10^5 and 10^6 cells/mL) were assessed in this study. Hormesis-like effects were found when microalgae grew with pMPs. The results showed that pMPs inhibited microalgae growth under a high concentration of microplastics tolerated by individual algal cell (low initial algae density) (up to −80.18±9.71%) but promoted growth when the situation was opposite (up to 15.27±3.66%). The contents of photosynthetic pigments including chlorophyll a, chlorophyll c and carotenoids showed resistance to pMPs stress under a low initial algae density and increased with time, but the opposite was true under a high initial algae density. Compared with the low initial algae density group, Qp received less inhibition, and NPQ (heat dissipation) also decreased under the high initial algae density. Under the low initial algae density, OJIP parameters such as S_m, N, Area, Pi Abs, ψ_o, φ_{Eo}, TRo/RC and ETo/RC were more perturbed initially and returned to the levels of the control group (without pMPs) over time, but they remained stable throughout the experiment at high initial algae density. These results show that microplastics in the marine environment may have different toxic effects on *P. tricornutum* at different growth stages, which is of great significance for understanding the impact of microplastics on marine microalgae and aquatic ecosystems.

1 Introduction

Plastic is a stable synthetic polymer that has been widely used in human life; however, it has become a widespread pollutant (Sun et al., 2020). Serious plastic pollution in marine environments caused by direct dumping has been observed (Horton et al., 2017). As previously reported, approximately 6300 million tons of plastic waste have been generated worldwide, and a proportion of this waste

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is decomposed into microplastics (MPs, < 5 mm) through physical and chemical actions (Thompson et al., 2004; Andrady, 2011; Geyer et al., 2017). Microplastics are mainly detected in areas with human activities (Wang et al., 2017), and they are primarily composed of polyethylene (PE), polystyrene (PS), and polypropylene (PP) (Zhang et al., 2015). The concentration of microplastics present in the ocean varies from region to region, and the units of microplastics concentration in the actual environment recorded in the literature are not uniform. As previously reported, in the seawater of Arabian Gulf, Qatar, the concentration of PE, PP, and PET microplastics with a size of < 5 mm was 0.0438–1.46 particles/m^2. The Maowei Sea is polluted by PES, PP, PE, PA, PS, POM, PU, and PBT microplastics, with concentrations of 1200–10100 particles/m^3 (Wang et al., 2021). In the freshwater environment, the concentration of microplastics can reach 30−50 mg/L (Su et al., 2016).

The long lifespan of plastic products is associated with the difficulty degrading these materials; thus, they are harmful to organisms, especially marine organisms (Lee et al., 2013). Small microplastics have a larger surface area and more easily absorb pollutants in the environment, and after ingesting these microplastics, the physiologic functions of marine organisms are impaired (Derraik, 2002; Von Moos et al., 2012; Lusher et al., 2013). MPs also affect marine organisms at the genetic, physiologic, and biochemical levels. For example, it has been found that PE and PS microplastics could interfere with enzyme synthesis of *Mytilus galloprovincialis* and even change gene expression (Avio et al., 2015). As an important primary producer in the ocean, microalgae play an important role in maintaining the marine ecological balance (Kaiser et al., 2011). Therefore, it is necessary to elucidate the relationship between MPs and microalgae to analyze the potential impact of MPs on the marine ecological environment.

At present, several studies have investigated the influence of microplastics on microalgae (Tang et al., 2021). It was found that polystyrene microplastics (10−100 mg/L) have a dose-dependent negative effect on the growth and photosynthesis of *Chlorella pyrenoidosa* with an initial algae density of 1.5×10^5 cells/mL (Mao et al., 2018). Studies found that 2 μm PS plastic microbeads did not have a significant effect on the growth of *Chaetoceros neogracile* and reported that aggregation between microalgae and microplastics occurred (Long et al., 2017). *Raphidocelis subcapitata* with a start density of 6×10^4 cells/mL showed growth promotion in the presence of 63−75 μm PE microplastics (25−100 mg/L) (Canniff and Hoang, 2018). In addition, it has been found that the toxicity of microplastics is related to the particle size of microplastics, with a smaller particle size corresponding to more obvious toxic effects on microalgae (Sjollema et al., 2016).

Diatoms are one of the important members of marine algae. They play an important role in the process of carbon fixation and are also an important food source for many planktons (Knuckey et al., 2006). *Phaeodactylum tricornutum* is a common marine diatom with complete sequenced genome (Nishida et al., 1997), and is often used as an experimental object in toxic researches (Hernandez and Dukelow, 1998). In addition, *P. tricornutum* can produce many high-value substances such as fucoxanthin, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and triglyceride (TAG) for biodiesel, so it plays an important role in aquaculture, bioenergy, medical treatment, and environmental protection. The growth of *P. tricornutum* is influenced by many factors including illumination, temperature, climate, and nutrients (Butler et al., 2020). To investigate the potential toxic effects of microplastics on marine microalgae, in this study, the effects of different concentrations of pristine polystyrene microplastics (pMPs) without any pretreatment on the growth, photosynthetic pigment content and chlorophyll fluorescence parameters of *P. tricornutum* was explored. These results provided a theoretical basis and data support to analyze the possible influence of polystyrene microplastics on marine microalgae, especially growth and photosynthesis.

2 Materials and methods

2.1 Microalgae cultures

The marine microalgal strain *P. tricornutum* Bohlin (FACHB-842) was purchased from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (Wuhan, China), and it was cultivated in f/2 medium prepared from artificial seawater (Harrison et al., 1980). The microalgae were cultured in a 500 mL Erlenmeyer flask placed in an incubator with a temperature of 25±1°C, light intensity of 65.15 μmol photons/($m^2 \cdot s$) and light-dark cycle of 12 h (Li et al., 2020). Thirty days was set as the incubation period, and the Erlenmeyer flask was shaken 2−3 times a day.

2.2 Microplastics and dosing methods

The testing methods used in this research complied with the Organization for Economic Cooperation and Development (OECD) testing guidelines. Pristine polystyrene microplastics were provided by the State Key Laboratory of Organic-Inorganic Composite Materials, College of Materials Science and Engineering, Beijing University of Chemical Technology. To characterize the composition of microplastics, XRD pattern was obtained using X-ray powder diffractometer (XRD-7000, Shimadzu, Japan) with a Cu-Ka irradiation (λ = 0.15406 nm). Data were recorded in the range of 5°−80° (2θ). The characteristic diffraction peak at $2\theta = 20^o$ indicated that the main component of the

microplastic was polystyrene (Fig. 1A). The particle size of the microplastics used in the experiment is 5μm (Fig. 1B), which was determined by a laser particle sizer analyzer (Mastersizer Micro, UK). To investigate the potential harm of microplastics, five groups of microplastics with concentrations of 0, 10, 20, 50, and 100 mg/L were added to 150 mL Erlenmeyer flasks containing f/2 medium. They were mixed by sonication for 20 min and the suspension was shown in Fig. S1. *P. tricornutum* Bohlin in the log phase (1.5×10^7 cells/mL) was added to the system to meet the experimental requirements of a low initial algae density (10^5 cells/mL) and high initial algae density (10^6 cells/mL).

2.3 Analytical methods

2.3.1 Algal growth

The density of microalgae was determined daily by counting cells on a hemocytometer under an optical microscope (XSZ-HS3, Chongqing Optoelectronic Instrument Co., Ltd., China), and daily details of algal densities are shown in Table S1 and S2 (Zhang et al., 2016). All samples were analyzed three times. The growth stimulation/inhibition ratios (SIR) were calculated by Eq. (1):

$$SIR(\%) = (C_X/C_0 - 1) \times 100\%, \quad (1)$$

where C_X refers to the algae density in the experimental group and C_0 refers to the algae density in the control group (0 mg/L microplastics). When the result is positive, it represents stimulation, whereas when the result is negative, it represents inhibition.

2.3.2 Photosynthetic pigments

The contents of photosynthetic pigments including chlorophyll a (Chl a), chlorophyll c (Chl c) and carotenoids (Car) were determined at 4 d and 7 d (Hong et al., 2009). During the determination, 20 mL of microalgae was suction filtered and 2 mL of 90% acetone and 2 drops of magnesium carbonate suspension were added. To extract the pigments, the samples were ground thoroughly, soaked for 2 h and then centrifuged at 2164 g for 10 min. The absorbance of the supernatant was determined at 470, 650, and 665 nm using a microplate reader (Multiskan-K3, Thermo, USA). All tests were repeated three times. The contents of pigments were calculated according to Eqs. (2)–(4) (Arnon, 1949; Grobe and Murphy, 1998):

$$Chla(\mathrm{mg/L}) = 11.47 \times Abs_{665} - 0.40 \times Abs_{650}\ , \quad (2)$$

$$Chlc(\mathrm{mg/L}) = 24.36 \times Abs_{650} - 3.73 \times Abs_{665}, \quad (3)$$

$$Car(\mathrm{mg/L}) = (1000 \times Abs_{470} - 2.05 \times Chla)/245, \quad (4)$$

where Abs_{470}, Abs_{650} and Abs_{665} refer to the absorbance of the supernatant (without microplastics) at 470, 650 and 665 nm, respectively.

2.3.3 Chlorophyll fluorescence parameters

To explore the underlying mechanism behind the effects of microplastics on the growth and photosynthetic pigment content of *P. tricornutum*, the changes in chlorophyll fluorescence parameters were evaluated. The chlorophyll fluorescence parameters were measured using a chlorophyll fluorometer (AquaPen-P100, Azure, USA) (Goiris et al., 2015). To adjust the test parameters, 10 mL of microalgae was placed in a 50 mL centrifuge wrapped with tin foil to protect microalgae from light. After dark adaptation for 15 min, the test was carried out and the saturating light was changed until Q_y moved between 0.65 and 0.7. The flash light was adjusted to maintain $5000 < F_t < 10000$. The intensities of actinic light and saturating light were 100 and 1500 μmol photons/($m^2 \cdot s$), respectively. Dark adaptation for 15 min was necessary before each test. The values of Fv/Fm, NPQ and Qp of each group were measured at 4 d and 7 d, and OJIP curves were drawn to obtain more detailed parameters including

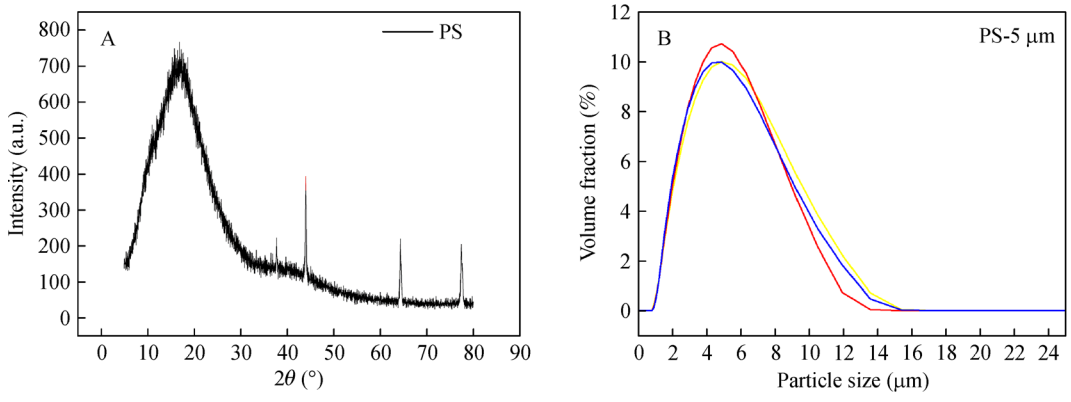

Fig. 1 The XRD patterns (A) and grading curve (B) of polystyrene microplastics.

Area, Sm, N, Pi Abs, ψ_o, φ_{Eo}, φ_{Do}, ABS/RC, TRo/RC, ETo/RC and DIo/RC. All the measurements were performed in triplicate. The physiologic meanings of the above parameters are shown in Table 1 (Kitajima and Butler, 1975; Bilger and Björkman, 1990; Wituszyńska et al., 2015).

2.4 Statistical analysis

All experiments were conducted in triplicate, and the results are expressed as the average±standard deviation. Analysis of variance with a *t*-test was applied to determine the significant difference at the 95% confidence level under different initial algae densities (Table S3 and S4) and microplastic concentrations using SPSS software (IBM SPSS Statistics 26, USA)

3 Results and discussion

3.1 Effects of microplastics on the growth of microalgae

The effects of pMPs on the growth of *P. tricornutum* Bohlin at the two initial algae densities are shown in Fig. 2. Microplastics inhibited the growth of microalgae with a low initial algae density (high microplastics concentration tolerated by individual cell) but promoted the growth under a high initial algae density (low microplastics concentration tolerated by individual cell), and the influence became more obvious as the microplastic concentration increased at 7 d.

Figure 2A shows that under the low initial algae density, microplastics at various concentrations always showed an inhibitory effect on the growth of *P. tricornutum* Bohlin throughout the experiment (0−7 d). A higher concentration of pMPs corresponded to a more obvious inhibitory effect. At 2−3 d, and the maximum inhibition was −76.06±7.54% at 2 d (100 mg/L) and −80.18±9.71% at 3 d (100 mg/L), which indicated that microalgae with low initial algae density were more sensitive to microplastics in the early stages of growth. The growth inhibition effect in the early stage affected the later stage of growth. At 7 d, although the nutrients were still sufficient, the growth of microalgae was still lower than that of the control group. The algae density of the 10, 20, 50, and 100 mg/L microplastics groups at 7 d was reduced by −29.78±2.63% ($p < 0.05$), −35.83±2.49% ($p < 0.01$), −42.83±1.49% ($p < 0.05$), and −65.29±1.98% ($p < 0.01$), respectively, compared to that of the control group (Fig. 2C). As the density of microalgae increased, microalgal adaptation was observed. Under a high initial algae density, the growth of *P. tricornutum* Bohlin was always promoted from 0 to 7 d at most concentrations of microplastics (except for 100 mg/L at 4–5 d). The microalgae were stimulated by the microplastics in the early stage of exposure (0–1 d in Fig. 2B), and the growth was better than the situation at 5−7d in Fig. 2A. The promotion trend gradually stabilized at 6 d (Fig. 2B). The promotion effects were positively related to the concentration of microplastics. At 7 d, the promotion effects of pMPs on the growth of *P. tricornutum* Bohlin increased with increasing microplastic concentration. Compared with the control group, the microalgae density of the 10, 50, and 100 mg/L microplastic groups at 7 d increased by 2.32±0.09%, 13.25±3.24% ($p < 0.05$), and 15.29±3.66% ($p < 0.05$), respectively (Fig. 2D), which indicated that under a high initial algae density, pMPs had a significant stimulatory effect on the growth of *P. tricornutum* Bohlin, and the effect was more obvious with increasing pMPs concentration. *P. tricornutum* can also proliferate quickly

Table 1 Physiologic meaning of the chlorophyll fluorescence parameters

Parameters	Description
Fv/Fm	Maximum photochemical efficiency of photosystem II (PS II)
NPQ	Non-photochemical quenching referring to the ability of photoprotection
Qp	Photochemical quenching representing the photosynthetic activity
Area	Area between fluorescence curve and Fm
S_m	Energy required to reduce Q_A completely
N	Times that Q_A was reduced
Pi Abs	Performance index based on absorbing quantum flux of light
ψ_o	Number of open reaction centers
φ_{Eo}	Quantum yield for electron transfer
φ_{Do}	Quantum yield for heat dissipation
ABS/RC	Total light energy RC absorbed
TR_o/RC	Energy used for electron transfer
ETo/RC	Energy to reduce Q_A
DIo/RC	Energy dissipated

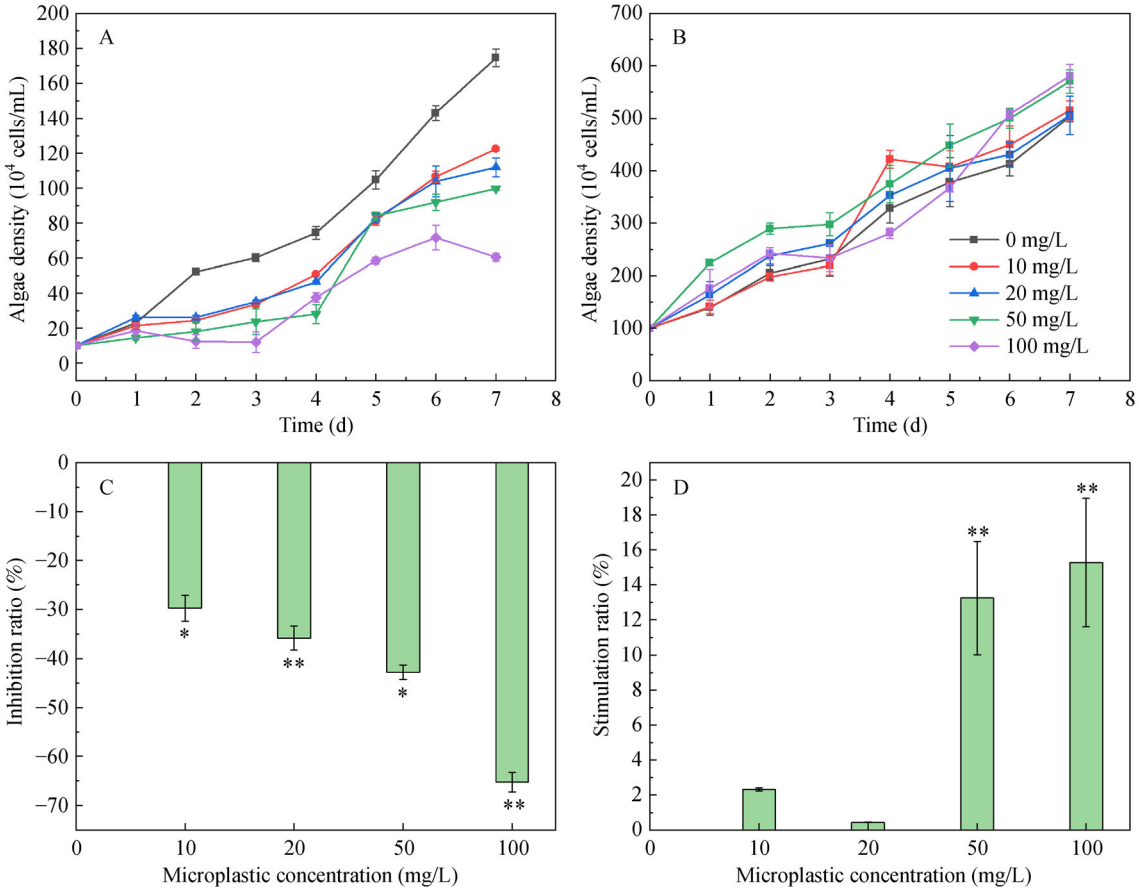

Fig. 2 Growth of *P. tricornutum* Bohlin exposed to pMPs. (A) Growth curves at a low initial algae density. (B) Growth curves at a high initial algae density. (C) Inhibition ratios at 7 d under a low initial algae density. (D) Stimulation ratios at 7 d under a high initial algae density. (average±standard deviation from triplicate experiments). * and ** represent the significant differences of $p < 0.05$ and $p < 0.01$ with the control group (0 mg/L microplastics), respectively.

under darkness-UV radiation two stage treatment (Qu et al., 2010; Cai et al., 2009), which was regarded as a compensatory growth when adverse conditions occur, and this is similar to the promotion effect of microplastics on *P. tricornutum* with high initial algae density in this study.

The studies mentioned below have found that some microplastics have negative effects on the growth of microalgae. Zhao et al. (2019) found that the density of *Karenia mikimotoi* (with an initial algae density of 10^6 cells/mL) decreased with increasing PVC microplastics concentration, and the maximum inhibition rate (IR) at 24 h was 45.8% (100 mg/L). The growth of *P. tricornutum* MASCC-0025 was inhibited after 96 h of exposure to 200 mg/L PP, PE, PET, and PVC microplastics, and MDA and SOD levels increased, CAT content decreased, and wrinkles and deformities were observed on the cell surface. These changes indicated that microplastics could cause oxidative damage to microalgae, reduce enzyme activity, and cause direct physical damage (Song et al., 2020). However, in this study, pMPs inhibited the growth of *P. tricornutum* Bohlin at the low initial algae density while promoting growth under the high initial algae density. Previous studies have also found similar results for the growth of *P. tricornutum* Bohlin with hygrothermal aging pMPs (10−100 mg/L) after a treatment for 20 min in an autoclave (121°C, 115 kPa) (Chen et al., 2020). Cunha et al. (2020) also found that low-concentration microplastics (0.5 mg/L PS) could promote the growth of *P. tricornutum* (with an initial algae density of 10^5 cells/mL) while high-concentration microplastics group (50 mg/L PS) showed slightly lower density than the control group. Since the effects of PS microplastics on *P. tricornutum* were not explored under higher initial algae density in the work mentioned above, and the concentration of microplastics tolerated by individual algal cell did not decrease, the promotion effect of 50 mg/L PS microplastics on microalgae was not observed, and the hormesis-like effects at a single initial algae density did not seem obvious. In fact, the concentration of microplastics in the environment varies widely (Wang et al., 2021; Su et al., 2016). The current researches focus on the toxic effects of relatively high-concentration microplastics, and the effects of microplastics with actual concentrations (trace concentrations) will be explored in the future.

Overall, when the initial algae density increased, the effect of pMPs on the growth of *P. tricornutum* Bohlin changed from inhibition to promotion. It was speculated that when the initial algae density increased, the number of microplastics exposed to individual algal cell decreased, which was equivalent to a reduction in the concentration of microplastics acting on the microalgae. At this time, the growth of algae was promoted by microplastics, which meant that low concentrations of microplastics tolerated by individual cell promoted the growth of *P. tricornutum* Bohlin while high concentrations of microplastics tolerated by individual cell inhibited growth. In other words, a hormesis-like effect was observed in this study. But what should not be ignored is that under the same initial algae density, different concentrations of microplastics did not show a direct hormesis-like effect. It might be because 10–100 mg/L microplastics were counted as high concentrations for individual microalgae cell at a low initial algae density, but after the initial algae density was increased by 10 times, this concentration range was counted as a low concentration for individual microalgae cell, so the results between different initial algae density groups constitute a hormesis-like effect. The hormesis effect was previously reported on freshwater microalga *Selenastrum capricornutum* when it was exposed to an allelochemical 2-methyl acetoacetate (EMA). As reported, when exposed to the same concentration of EMA, the growth of *S. capricornutum* was facilitated under high initial algae density, but was inhibited at low initial algae density (Hong et al., 2009). The effect was also found in *Chlorella* sp. L38 mixed with microplastics (Song et al., 2020). Low-dose microplastics triggered the self-protection mechanism of microalgae (with a high initial algae density of 10^6 cells/mL), which led to promotion. Conversely, high-dose microplastics exceeded the tolerance of the microalgae (with a low initial algae density of 10^5 cells/mL), thus inhibiting the growth of the microalgae (Jalal et al., 2021). A similar pattern could also be found in the high initial algae density group. For example, under 100 mg/L microplastics, the growth of microalgae before five days of cultivation was close to or lower than that of the control group due to the small number of algae cells and high concentration of microplastics. Nevertheless, the growth of microalgae at 6–7 d was significantly higher than that of the control group, indicating that after the increase in cell number, fewer microplastics were allocated to individual algal cells than before and the growth of microalgae was promoted (Fig. 2B).

3.2 Contents of photosynthetic pigments in microalgae mixed with microplastics

Since the growth of microalgae is related to photosynthesis in organisms, photosynthetic pigment contents are also important indicators to assess the toxic effects of microplastics. To determine the potential reasons for the changes in the growth of *P. tricornutum* Bohlin caused by pMPs, the photosynthetic pigment (Chl a, Chl c and Car) contents were measured. The results showed that the high concentration of microplastics at the low initial algae density group reduced the contents of the three pigments in the early stage but then the content levels recovered, while the opposite was true under the high initial algae density (Fig. 3).

Under a low initial algae density, chlorophyll a, chlorophyll c and carotenoids showed obvious resistance to pMPs stress at 4 d. The pigment contents in most of the experimental groups were higher than that of the control group, and the maximum contents of Chl a, Chl c and Car were $(25.92\pm0.92) \times 10^{-8}$, $(11.45\pm0.39) \times 10^{-8}$ and $(12.46\pm0.22) \times 10^{-8}$ g/cell, respectively ($p < 0.01$) (50 mg/L in Fig. 3A). However, 100 mg/L microplastics inhibited the pigment contents (Fig. 3A). As the treatment time extended, the contents of Chl a, Chl c and Car continued to increase at 7 d (Fig. 3B), and the pigment contents of the 100 mg/L concentration group were significantly higher than those of the other groups ($p < 0.01$). Although the growth of microalgae was inhibited at a low initial algae density, the pigment contents still increased, which could be regarded as an adaptation or resistance mechanism of microalgal cells. Similar results were also found during *P. tricornutum* exposure to 0.5 mg/L PS and polymethyl methacrylate (PMMA), and the contents of Chl a and Car increased in the first 6 days compared to that of the control group (Cunha et al., 2020).

The contents of Chl a, Chl c, and Car decreased slightly and then increased with increasing pMPs concentration at 4 d under a high initial algae density. When the pMPs concentration was 10 mg/L, the minimum content of the three pigments (Chl a, Chl c, and Car) was $(13.96\pm0.12) \times 10^{-8}$, $(5.59\pm2.65) \times 10^{-8}$ and $(6.74\pm0.01) \times 10^{-8}$ g/cell, respectively ($p < 0.05$) (Fig. 3C). This result might be related to the shading effect of a single cell under the same light intensity (Zhang et al., 2017), and the decrease in chlorophyll content might be caused by oxidative stress (Xiao et al., 2020). Due to the increase in the initial algae density, the amount of light available to individual algal cells was reduced; thus, the pigment contents were slightly reduced in the early stage under the high initial algae density. As the time period extended, the pigment contents gradually recovered to the level of the control group. At 7 d, the contents of Chl a, Chl c and Car were not significantly different among the groups and were even slightly higher than those of the control group at low microplastic concentrations. When the pMPs concentration was 20 mg/L, the maximum contents of Chl a, Chl c and Car were $(26.13\pm1.37) \times 10^{-8}$, $(10.71\pm0.11) \times 10^{-8}$ and $(28.14\pm0.24) \times 10^{-8}$ g/cell, respectively. However, at high microplastic concentrations (50 and 100 mg/L), the contents of the three pigments were inhibited compared with the control group (Fig. 3D), which implied that low

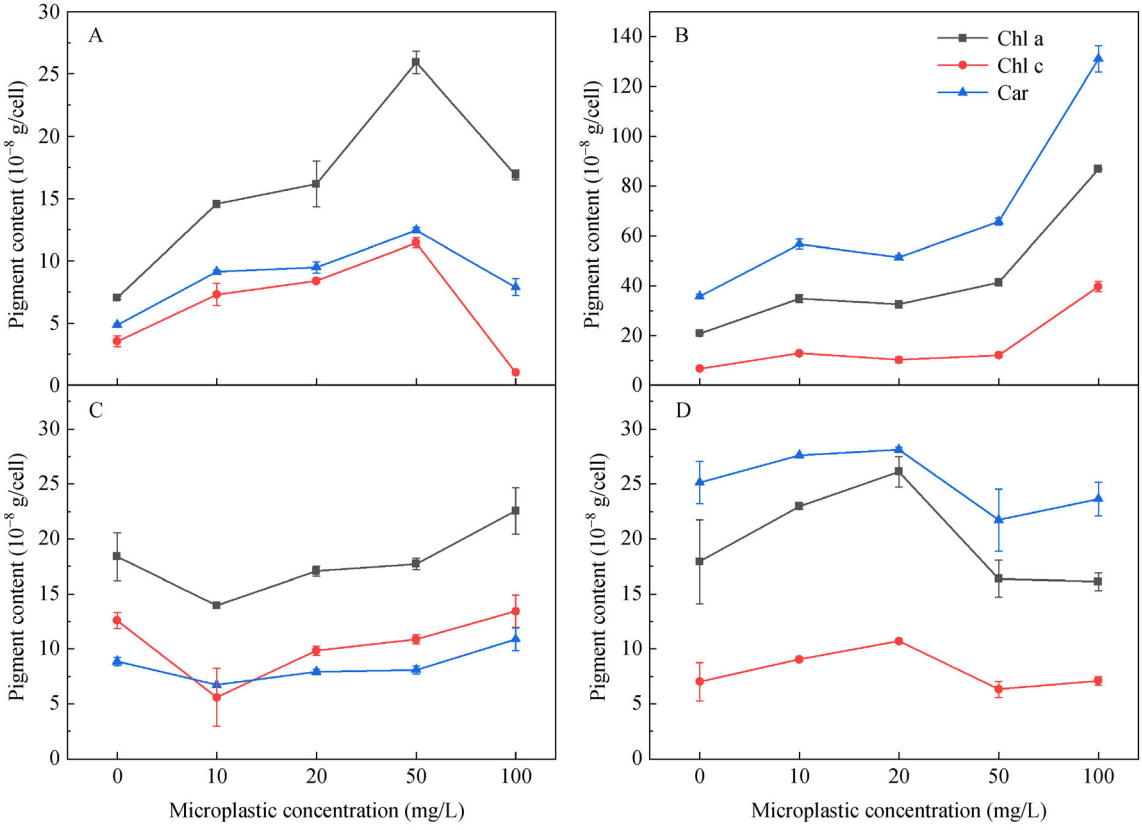

Fig. 3 Pigment changes in *P. tricornutum* Bohlin exposed to polystyrene microplastics under different initial algae densities. (A) Low initial algae density at 4 d. (B) Low initial algae density at 7 d. (C) High initial algae density at 4 d. (D) High initial algae density at 7 d. (average±standard deviation from triplicate experiments).

concentrations of microplastics were beneficial to increasing the photosynthetic pigment contents while high concentrations of microplastics had a negative effect on these contents. This finding was different from the effects of hygrothermal aging pMPs on the contents of the three pigments of *P. tricornutum*. The main target of hygrothermal aging pMPs was Chl a, and it had a suppressive effect, while the other two pigments had slight promoting effects (Chen et al., 2020). Besseling et al. (2014) found that 103 mg/L nano-PS caused a decrease in the chlorophyll content of *Scenedesmus obliquus*, which showed that the particle size and pretreatment of the microplastics influenced the outcomes.

3.3 Characteristics of the chlorophyll fluorescence parameters of microalgae after exposure to microplastics

To investigate the response of the photochemical process of microalgae in PSII, chlorophyll fluorescence parameters including Fv/Fm, NPQ, Qp, and OJIP curves were estimated. These parameters can reflect the changes in energy exchange and electron transfer during photosynthesis (Wituszyńska et al., 2015). The value changes of Fv/Fm, NPQ and Qp are shown in Fig. 4-6. The results showed that the photosynthetic activity (Qp) of the low initial algae density group decreased significantly in the early stage and then returned to the control level, while at the high initial algae density, the heat dissipation (NPQ) was significantly reduced and the electron transfer (ETo/RC) showed a negligible change.

Under the low initial algae density, Fv/Fm was disturbed slightly at 4 d, and the minimum value was 0.53±0.07 at 10 mg/L (87.46% of control). Increases in Fv/Fm of 2.48% and 2.97% in the 50 and 100 mg/L microplastic groups were observed, respectively (Fig. 4A). At 7 d, the Fv/Fm value of the microplastic group remained near the control (Fig. 4B). The NPQ also suffered from interference. At 4 d, it reached the minimum value of 0.69±0.21 (77.97% of control) when the microplastic concentration was 10 mg/L. The NPQ values of the 20, 50 and 100 mg/L microplastic groups increased by 2.82%, 1.98% and 1.13%, respectively (Fig. 4A). When the time was extended to 7 d, NPQ was promoted by exposure to microplastics at various concentrations. When the concentration of microplastics was at its maximum (100 mg/L), the maximum NPQ value of 1.06±0.11 was observed, and it represented an increase of 19.77% (Fig. 4B). The values of Qp at various concentrations of microplastics were lower than those of the control at 4 d. When the microplastic concentration was 20 mg/L, the Qp reached the minimum value of 0.16±0.03 and the inhibition ratio was 71.82% (Fig. 4A). At 7 d, the inhibitory effect of each microplastic concentration group

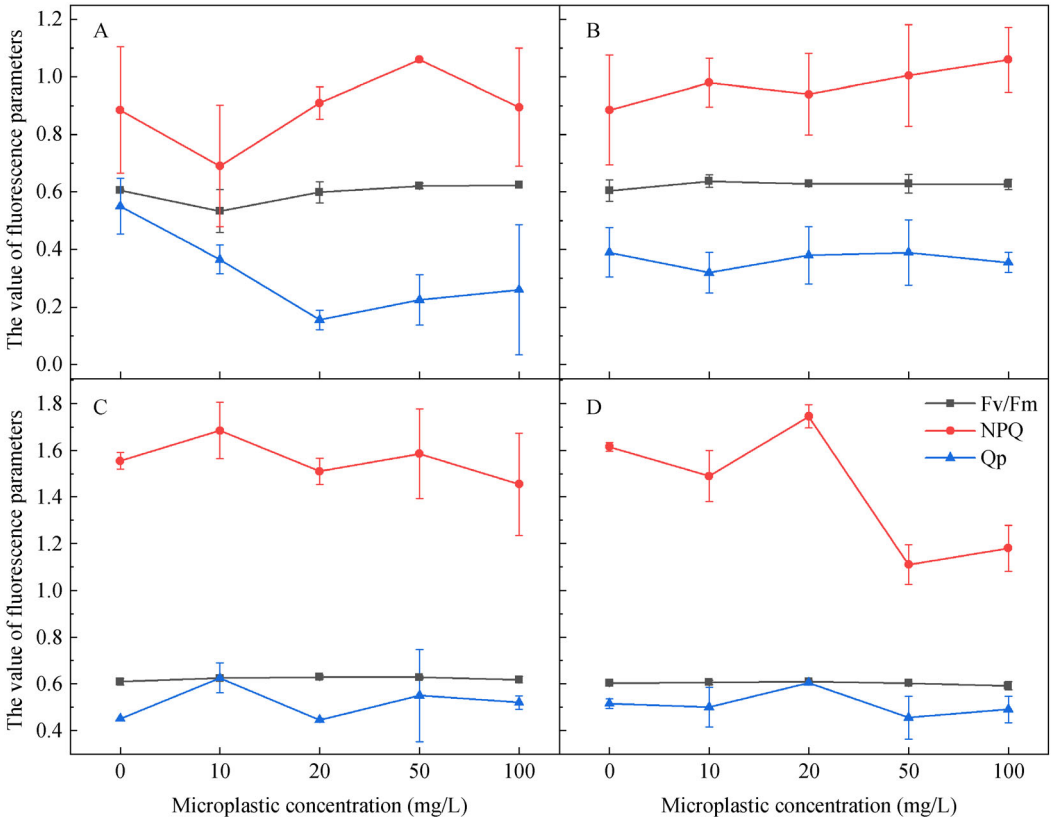

Fig. 4 Changes in Fv/Fm, NPQ and Qp of *P. tricornutum* Bohlin exposed to polystyrene microplastics. (A) Low initial algae density at 4 d. (B) Low initial algae density at 7 d. (C) High initial algae density at 4 d. (D) High initial algae density at 7 d. (average±standard deviation from triplicate experiments).

on Qp was weakened, and at 10 mg/L, the lowest Qp value of 0.32±0.07 was observed, which was 17.95% less than that of the control group, and the values of the remaining concentration groups were close to that of the control (Fig. 4B).

Under the high initial algae density, there was only a slight increase in Fv/Fm at 4 d, and the Fv/Fm stimulation ratios of the 10, 20, 50, and 100 mg/L groups were 2.46%, 3.28%, 3.04% and 1.48%, respectively. At 7 d, the values were still stable. The maximum inhibition ratio was −1.83% when the microplastic concentration reached 100 mg/L (Fig. 4D). The values of NPQ at 4 d showed slight fluctuations. The SIR of NPQ values in the 10, 20, 50 and 100 mg/L microplastic concentration groups was 8.36%, −2.89%, 1.93%, and −6.43%, respectively (Fig. 4C). However, NPQ was significantly inhibited ($p < 0.01$) at 7 d due to the high concentrations of microplastics (>20 mg/L), and the values of NPQ decreased by 31.27% and 26.93% with 50 and 100 mg/L microplastics, respectively (Fig. 4D). At each microplastic concentration, the values of Qp showed a similar trend to that of NPQ at 4 d (Fig. 4C). At 7 d, the synchronous variation of Qp and NPQ was still observed, but the variation range of Qp was small. The minimum value of Qp was 0.49±0.06 (96% of control), which was observed in the 50 mg/L microplastic group (Fig. 4D).

The value of Fv/Fm represents the maximum quantum yield of of PSII photochemistry and the potential photosynthetic capacity (Baker, 2008). Qp is often used to evaluate the photosynthesis capacity of plants, which is proportional to the quantum yield of acyclic electron flow (Genty et al. 1989). A high Qp value means that more PSII primary quinone electron acceptor (Q_A) is maintained in oxidation. The increase in NPQ is used to dissipate excess photon energy. Studies have found that *P. tricornutum* cannot make good use of light energy under CO_2 limitation, and NPQ will be significantly increased in order to avoid light damage, which is related to changes in pH, carotenoids and some gene products (Waring et al. 2010; Shimakawa et al. 2017).

In this study, the value of Fv/Fm did not change, indicating that the maximum photochemical efficiency under dark adaptation conditions had not changed; that is, the potential photosynthesis capacity was not affected, but the change in Qp indicated that the actual photochemical efficiency under light adaptation conditions was affected. Although there was no obvious association between the Qp and Fv/Fm changes, some correlations were found between the Qp and NPQ changes. Under the low initial algae density, the values of NPQ and Qp changed almost in the opposite directions based on the concentration. Qp decreased significantly at 4 d, and heat dissipation (NPQ)

increased compared to the control group (Fig. 4A). The above phenomena were more obvious under the action of high concentrations of microplastics. The decrease in Qp showed that at the low initial algae density, microplastics hindered the downstream part of photosynthesis and the proportion of open photochemical electronic gates decreased. Moreover, heat dissipation (NPQ) increased, which was regarded as photoprotection for microalgae, indicating that the two processes were in direct competition. At 7 d, although the changes in the two parameters were still opposite, they tended to be stable due to the adaptation mechanism (Fig. 4B). However, under the high initial algae density, the changes in the two parameters were almost synchronized, which might imply that processes other than heat dissipation or photochemical action were responsible for dissipating energy. Fortunately, Qp varied only slightly with the microplastic concentration, and NPQ also showed the same decreasing trend under the high initial algae density (Fig. 4D). This result suggested that the photosynthetic performance at a high initial algae density was stronger than that at a low initial algae density. In summary, hormesis-like effects on the photosynthesis of *P. tricornutum* Bohlin may occur when exposed to microplastics.

When plants are subjected to abiotic and biotic stress, Fv/Fm values generally decrease (Baker, 2008). Previous studies found that when *P. tricornutum* Bohlin grew with hygrothermal aging pMPs, the values of Fv/Fm obviously decreased under a low initial algae density and increased at a high initial algae density. The hygrothermal aging microplastics promoted Qp significantly under the high initial algae density at 7 d. NPQ remained reduced throughout the experiment, except at 7 d under the high initial algae density (Chen et al., 2020). The maximum inhibition rate of 100 mg/L PVC microplastics for the Fv/Fm of *Karenia mikimotoi* was 17.1% (Zhao et al., 2019). Fv/Fm initially decreased and then increased with time when *Chlorella pyrenoidosa* grew with PP and PVC (Wu et al., 2019). However, there was no significant change in Fv/Fm in this experiment, which was obviously different from the results mentioned above, indicating that pMPs had different mechanisms of photosynthesis in microalgae.

OJIP curves and related parameters were measured to understand some details of photosynthesis of *P. tricornutum* growing with the microplastics. OJIP fluorescence transients and the values of Area, S_m, N, PiAbs, ψ_o, φ_{Eo}, φ_{Do}, ABS/RC, TRo/RC, ETo/RC and DIo/RC in the microalgae exposed to different concentrations of microplastics are displayed in Figs. 5 and 6. The values of the index in Fig. 6 are the ratios of the experimental group

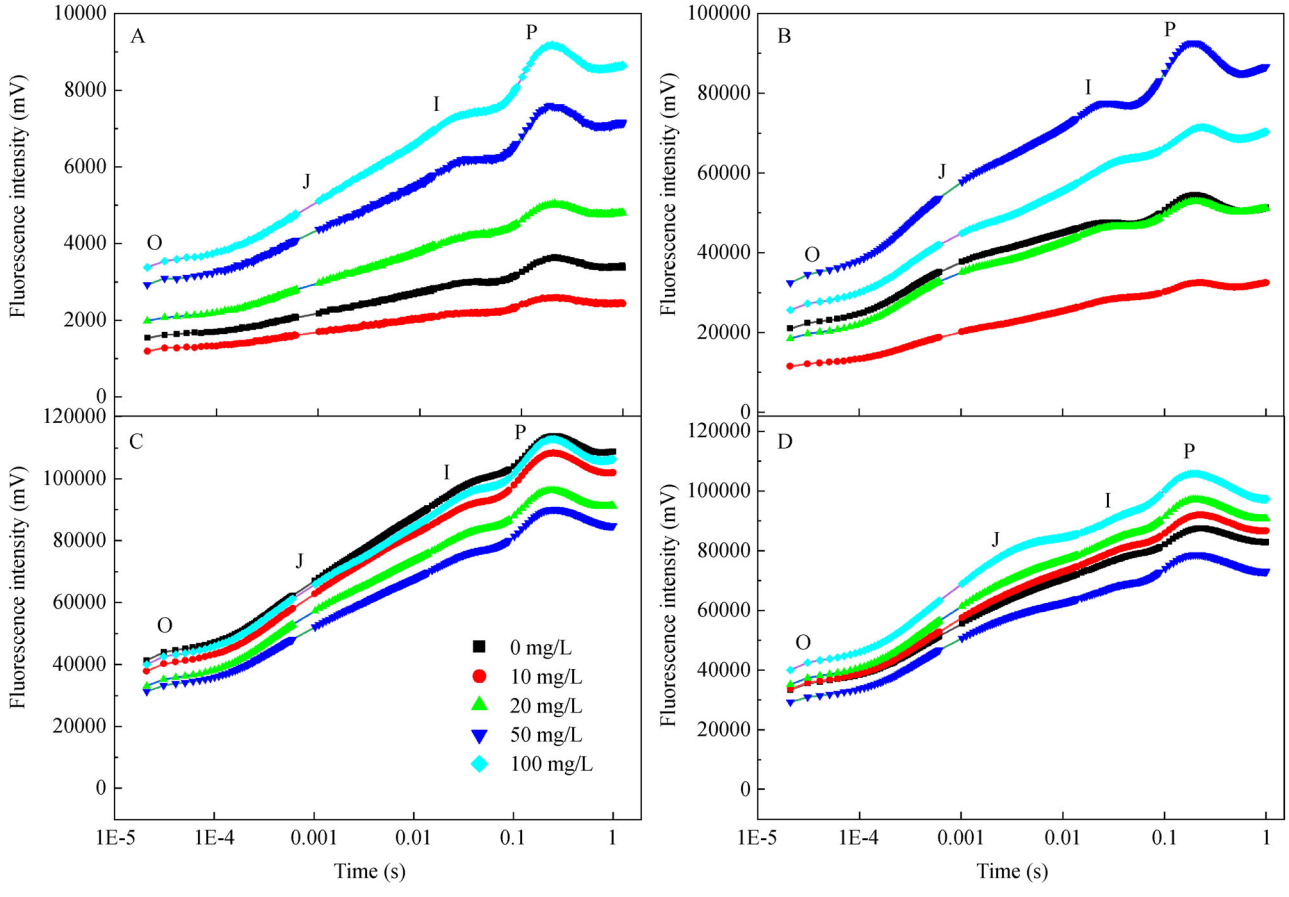

Fig. 5 OJIP fluorescence transients of *P. tricornutum* Bohlin at different polystyrene microplastic concentrations. (A) Low initial algae density at 4 d; (B) Low initial algae density at 7 d; (C) High initial algae density at 4 d; (D) High initial algae density at 7 d.

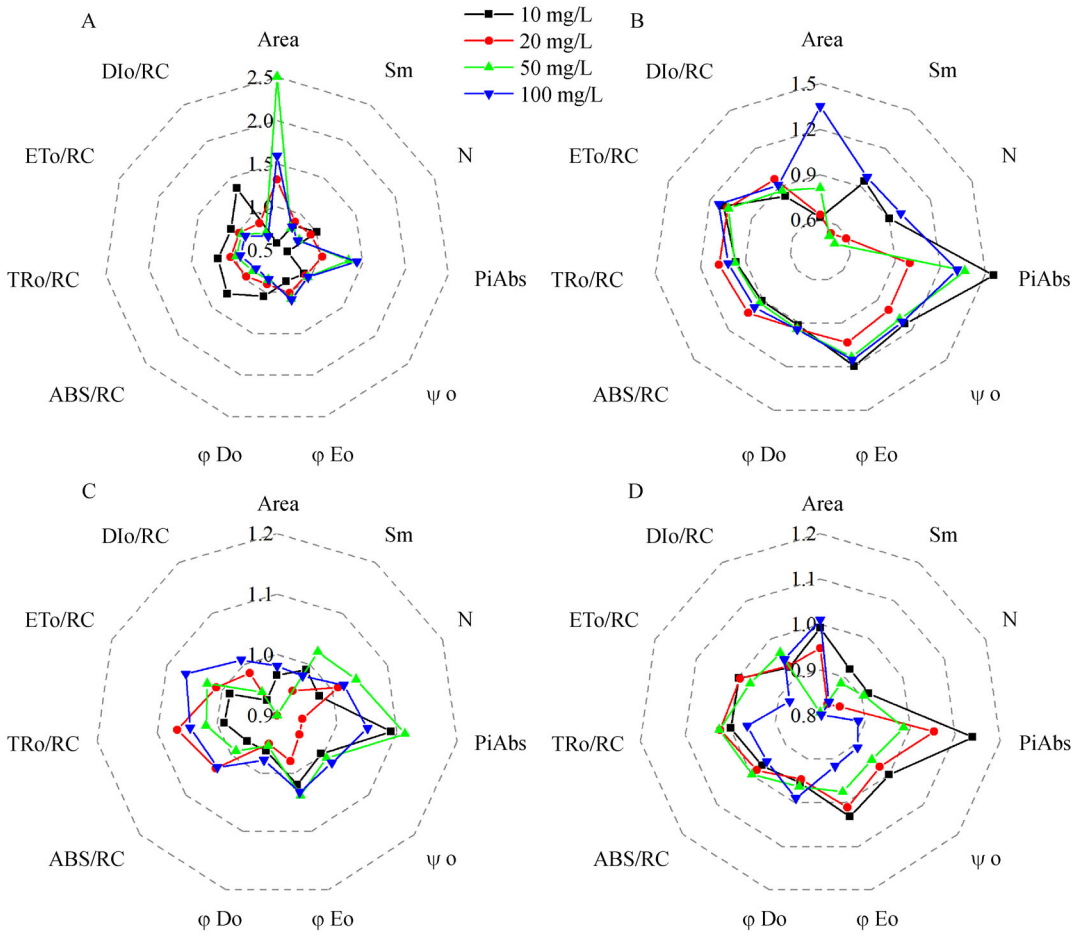

Fig. 6 Changes in the OJIP parameter values of *P. tricornutum* Bohlin at different polystyrene microplastic concentrations. (A) Low initial algae density at 4 d; (B) Low initial algae density at 7 d; (C) High initial algae density at 4 d; (D) High initial algae density at 7 d.

divided by the control group. If the value is more than 1, then microplastics promoted the parameter relative to the control (0 mg/L); otherwise, microplastics inhibited the parameter.

The OJIP curve reflects the process of electrons transfer in the reaction center of PSII from Q_A to Q_B and then to PQ (Strasser and Strasser, 1995). The change trend of the OJIP curve of the microplastics group was basically the same as that of the control group, and the occurrence time of the characteristic points (O, J, I and P) was also the same as the control. Under the action of microplastics, the fluorescence value of the OJIP curve moved up and down around the control group as a whole, that is, the values of Fo and Fm increased and decreased in the same way, and this movement does not show a concentration correlation, which corresponds to the unchanged Fv/Fm (1-Fo/Fm) (Fig. 5). However, in general, compared with the high initial algae density group, the movement range was larger at low initial algae density, and the slope of the curve increased significantly at 50 and 100 mg/L group, indicating that the reduction of Q_A was faster, and the opposite was true in the 10 mg/L group (Fig. 5A and 5B). This shows that *P. tricornutum* have a strong struggle to deal with higher concentration microplastics (Zhu et al., 2005). While at high initial algae density, the magnitude of this shift and slope change was significantly reduced (Fig. 5C and 5D). This indicated that under low initial algae density (high concentration of microplastics tolerated by individual cell), the photosynthesis of *P. tricornutum* was more disturbed.

At 4 d under the low initial algae density, most of the OJIP parameters, especially in the 10 mg/L group, were relatively unstable. The values of TRo/RC and ETo/RC were promoted with 10 mg/L microplastics but slightly inhibited when exposed to 50 and 100 mg/L microplastics, indicating that electron transport with high concentrations of microplastics was not as smooth as with low concentrations. DIo/RC and ABS/RC also showed a similar regulatory effect, and they multiplied more than TRo/RC and ETo/RC. When rice grew under abiotic pressure, both DIo/CSo and ABS/CSo increased at the same time, and the degree of increase was greater than that of TRo/CSo (Faseela et al., 2020). This finding might imply that the extra energy absorbed did not increase the

rate of electron transfer but was dissipated as heat or fluorescence. Contrary to the rule mentioned above, the values of Area and Pi Abs changed in reverse. In the 10 mg/L group, Area and Pi Abs were 57.9% and 62.3% of the control, respectively, but in the 50 mg/L group, they reached the maximum (250% and 133% of the control, respectively) (Fig. 6A). With the increase in microalgae density at 7 d, the parameters of each group were closer to those of the control group except for Area and Pi Abs. Some parameters such as quantum yield for electron transfer (φ_{Eo}) and the number of active reaction centers (ψ_o) were slightly higher than those of the control group (119% and 113% of the control) (Fig. 6B).

Under the high initial algae density, almost all the parameters were close to the control at 4 d, even Area and Pi Abs. The values of ABS/RC, TRo/RC and ETo/RC in the 20 and 100 mg/L concentration groups were all greater than 1, which indicated that the electron transfer efficiency of microalgae with a high initial algae density was slightly improved under the stimulation of low-dose microplastics, while the photosynthetic capacity was not greatly disturbed (Fig. 6C). At 7 d, most of the indicators were still maintained close to the control, and only the values of S_m and N in the 100 mg/L group were significantly lower than those in the control group (Fig. 6D). The same results were also found in the 20 and 50 mg/L groups at 7 d under the low initial algae density. The S_m and N values of *P. tricornutum* Bohlin mixed with hygrothermal aging pMPs also changed significantly under different algae densities (Chen et al., 2020). The decrease in S_m indicated that the electron transporter on the PSII acceptor side decreased, which led to a decrease in the probability of the captured light energy transferring electrons to the acceptor exceeding Q_A in the electron transport chain (φ_{Eo}). At the same time, the decrease in the number of reductions of Q_A indicated that the ability of Q_A to transfer electrons was diminished, which was manifested in the decreased opening reaction center number (ψ_o). This finding implied that prolonged exposure to microplastics might cause a reduction of electron mediators, which in turn affected subsequent electron transfer.

According to the large fluctuations in OJIP parameters at 4 d under the low initial algae density, it was speculated that microplastics might affect energy exchange and electron transfer in the photosynthetic process of *P. tricornutum*. With increased time and algae density, the microalgae could gradually overcome the stress of microplastics and the photosynthetic capacity was almost unaffected, which was consistent with the control. The OJIP parameters did not show a significant hormesis-like effect that could promote growth at low concentrations of microplastics but rather exhibited inhibition at high concentrations; even so, these parameters obtained under the low initial algae density (high-dose microplastics) were more unstable than those under the high initial algae density (low-dose microplastics) when exposed for a short time. This finding was more evident in Area and Pi Abs. Moreover, at the low initial density, the values of Area and Pi Abs in the 20−50 mg/L microplastic groups were exceptionally higher than those of the control. The area (Area) between the fluorescence induction curve and Fm could be used to evaluate the size of the reduced plastoquinone pool on the reducing side of PSII. An increase in Pi Abs also indicated a better photosynthetic capacity (Faseela et al., 2020). These results were not consistent with the phenomenon that the growth of microalgae was inhibited under a low initial algae density, but it might represent a stress response of microalgae for survival under microplastic exposure in the early stage.

4 Conclusions

pMPs had hormesis-like effects on the growth of *P. tricornutum* Bohlin. An inhibitory effect of pMPs on *P. tricornutum* Bohlin was observed under a low initial algae density (high concentration of microplastics to individual cell), and the inhibition ratio increased with the concentration of microplastics; however, pMPs stimulated the growth of *P. tricornutum* Bohlin at a high initial algae density (low concentration of microplastics to individual cell), and the promotion effects became increasingly obvious with increasing microplastic concentration. The photosynthetic pigment content of low initial algae density group exhibited stress resistance to pMPs and then increased continuously. At a high initial algae density, the pigment content increased initially and then decreased slightly under the effect of a high concentration of pMPs. Qp decreased significantly at 4 d under the low initial algae density and then returned to the control level. NPQ increased initially and then decreased sharply after the initial increase in algae density. Electron transfer and energy exchange were more perturbed at 4 d under the low initial density than under the other conditions. In general, the growth and photosynthetic performance of *P. tricornutum* under low initial algae density were poor compared with those of high initial algae density group, which indicated that microplastics with different concentration ranges might have different toxic effects on microalgae at various growth stages. The changes in photosynthetic electron and energy transfer might be the mechanisms underlying these results.

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